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(54) Title: NOVEL DERMATAN SULFATE AND HEPARIN OLIGOSACCHARIDES HAVING ANTIATHEROSCLEROTIC ACTIVITY		
(57) Abstract The invention comprises: mucopolysaccharides consisting of dermatan sulfate fragments in which the unsulfated uronic acid moieties have been opened, by oxidation of the C(2)-C(3) bonds with periodic acid, and the process for the preparation thereof; oligosaccharides obtained from heparin by a depolymerization process, which oligosaccharides have molecular weights ranging from 3.500-1.000 daltons; pharmaceutical compositions having antiatherosclerotic, arterial antithrombotic, anti-platelet aggregation activities, containing the above mentioned mucopolysaccharides and oligosaccharides.		

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NOVEL DERMATAN SULFATE AND HEPARIN OLIGOSACCHARIDES
HAVING ANTIATHEROSCLEROTIC ACTIVITY

The various pharmacological activities of heparin, heparan sulfate, dermatan sulfate are almost always related to the presence of characteristic structural blocks which are contained in the longest polysaccharide chain. As an example, the more important requirement for the anticoagulant activity of heparin consists in the presence of a specific pentasaccharide containing a D-glucuronic acid residue and only one trisulfated D-glucosamine residue (Thunberg L. et al., Carbohydr. Res., 100, 393, 1982; Casu B. et al., Bioch. J., 197, 599, 1981; Choay J., Biochem. Biophys. Res. Comm., 116, 492, 1983). This sequence is the real binding site for Antithrombin III (AT III). Heparin co-factor II (HC II) is activated by an oligosaccharide consisting of at least eight monosaccharides, which is contained in dermatan sulfate (Tollefsen D.M. et al., J. Biol. Chem., 262, (19), 8854, 1986).

Therefore, a specific monosaccharide sequence, contained in the larger structure of the various polysaccharides, seems to be essential for the activation of specific active sites.

Now, it has surprisingly been found that increasing the flexibility of dermatan sulfate polysaccharide chain the probability of binding with plasma proteins raises and accordingly the pharmacological activity of dermatan sulfate itself increases.

Periodate is known to cleave the bond between the

carbons bearing two unsubstituted vicinal hydroxy groups. In dermatan sulfate almost all the C(2) and C(3) positions of uronic acids have two unsubstituted trans hydroxyls, and therefore said atoms, are under the above cited condition, consequently periodate anion cleaves the C(2)-C(3) bond oxidizing the respective carbon atoms to aldehydes, resulting in the cleavage of the uronic acid saccharide ring. Only uronic acid moieties having an OSO₃ group at C(2) or C(3) remain unaffected, said moieties being a minor part. Therefore the whole macromolecular chain, when subjected to oxidation by periodate ion, acquires a different conformation than that of the starting dermatan. The aldehyde groups at C(2) and C(3) can be reduced to primary alcohols by means of sodium borohydride, or they can be oxidized. Anyhow, dermatan chain turns out to be more labile to acid environment and it can undergo degradation to low molecular weight oligomers (Smith degradation) or even to the constituent units (see scheme 1).

A wide molecular weight range can be obtained by suitably adjusting the hydrolysis conditions. The obtained fractions can be selected on the ground of increasing charge densities on ion exchange resins.

Such a succession of reactions has already been applied to dermatan sulfate for analytical purposes (Fransson L.A. et al., Carbohydr. Res., 36, 349, 1974). Nevertheless, the structure of the obtained oligomers had not been demonstrated neither the surprisingly increased antithrombotic activity thereof, compared with that of the starting dermatan, had been evidenced. Equally

surprisingly, dermatan sulfate open in C(2)-C(3) proved to have poor or no affinity for heparin cofactor II. The antithrombotic action of dermatan sulfate likely depends not only on the ability thereof to activate heparin cofactor II (Tollefsen D.M. et al., J. Biol. Chem., 257, 2162, 1982; Tollefsen et al., J. Biol. Chem., 258, 6713, 1983) but also on still unknown factors and/or mechanisms.

Patents or patent applications are known claiming the pharmacological activities of dermatan sulfate or fragments or derivatives thereof; nevertheless the pharmacological activities of "open" dermatan sulfate and the fragments thereof have not been disclosed up to now.

EP-A-0 097 625 claims a substantially pure dermatan sulfate having antiedemic and anticongestive activities. EP-A-0 199 033 and 0 238 994 claim pure dermatan sulfates having antithrombotic activity. GB-Patent 2 098 232 generically claims an artificially polysulfated, chemically depolymerized chondroitin sulfate having a pharmacological action.

PCT/EP/00291 claims fractions from dermatan sulfate which has been subjected to depolymerization to 5-6 Kd molecular weight, which fragments have antithrombotic activity and improved bioavailability.

EP-A-0 208 623 generically claims polysaccharides, and therefore also dermatan sulfate (claim 6) having low molecular weights (below 10 Kd), active on connective tissue pathologies.

Japan Kokai JP 82.40502 (CA 97, 28639, 1982) claims fractions from dermatan sulfate which has been

depolymerized in DMSO at pH \leq 2 and resulfated with H_2SO_4 .

French Patent n. 2 584 728 claims the sulfation of all the glucosaminoglycans, and therefore also of polysulfated dermatan sulfate in pyridine with pyridine-sulfotrioxide.

The present invention relates to a dermatan sulfate having open unsubstituted uronic acid moieties and the uronic acid which have a $O-SO_3$ group at C(2) or C(3) unchanged. Moreover, the present invention also relates to "open" dermatan sulfate fragments.

Surprisingly said polysaccharides have antiatherosclerotic, antithrombotic and thrombolytic activities, said activities being even more than 4 times higher than those of the natural dermatan sulfate from which they derive.

The present invention also relates to oligosaccharides from heparin of molecular weight ranging from 3.500 to 1.000 daltons. Such oligosaccharides have no anticoagulant activity neither affinity for antithrombin III (AT III), they have in vivo antiatherosclerotic activity, also by oral administration, they inhibit platelet aggregation and block spontaneous muscle contraction. The latter activity is related to the proliferation of subendothelial smooth muscle cells.

The present invention also relates to the process for the preparation of very low molecular weight heparin oligosaccharide fragments. All the heparin fractions or fragments known up to now and described in the prior art are characterized by poor anticoagulant (APTT) and

antithrombotic activities related to high AT III affinity. Anyhow, all the hitherto described processes for heparin depolymerization yield fragments of lower molecular weight than those of heparin, but having
5 different structural characteristics.

Surprisingly, the heparin oligosaccharides of the present invention retain the heparin structure, except for molecular weight, and moreover have antithrombotic activity in experimental venous thrombosis models and,
10 surprisingly, also in the arterial ones.

A number of processes for the depolymerization of heparin have been described in the prior art; products can be obtained differing for structural characteristics peculiar to each process. Said characteristics
15 distinguish the known compounds from the ones of the present invention.

US Patent 4,303,651 (corresponding to EP-A0 014 184) claims an oligosaccharide having 14-18 saccharide units and molecular weight of about 3.600-4.800 daltons, obtained by deaminative depolymerization with nitrous acid.
20 The main component of said oligosaccharide is the disaccharide L-iduronosyl-2-O-sulfate-D-glucosamine-N, 6-disulfate, and unsulfated iduronic acid is present at a position distant 3-5 saccharide units from the not reducing
25 end. The oligosaccharide contains no glucuronic acid.

US Patents 4,500,519 and 4,351,938 claim oligosaccharides obtained by depolymerizing heparin with nitrous acid: the reducing ends thereof consist of anhydromannose which can be reduced to anhydromannitol or oxidized to
30 D-mannonic acid.

US Patents 4,401,662 and 4,474,770 claim an oligosaccharide having high antithrombotic activity, high affinity to AT III, extremely high anti-Xa activity, which oligosaccharide consists of no more than 8 monosaccharides and is obtained by depolymerization with nitrous acid or heparinase. Its structure contains glucosamine having a OSO_3 group also at the 3-position.

EP-A- 0 048 231 claims an oligosaccharide containing 4-8 monosaccharides, characterized by the presence of a 3-O-sulfated glucosamine and by a reducing end consisting of 2,5- anhydro-D-mannose.

The depolymerizations described in all the above cited patents involve the acetalic linkage between the amino sugar and the uronic acid. Therefore, the obtained oligosaccharides are always compounds having a whole number of disaccharides. Moreover, where the cleavage takes place, a sulfur loss always occurs.

US Patent 4,281,108 discloses a low molecular weight heparin obtained by N-desulfation through the intermediate heparamine, depolymerization by heating in acid medium, in the presence of an oxidizing agent, and subsequent resulfation. This process causes substantial changes in the heparin structures, which cannot be restored even by resulfation.

EP-A-40 144 discloses a process for alkali hydrolysis of heparin.

In EP-A-44 228 hydrolysis always occurs on alkyl or aryl esters at the heparin carboxy group, and gives oligomers of mean molecular weight of 2.000-9.000 daltons.

Both said latter processes yield oligomers having an

end Δ -4,5-unsaturated uronic acid, through β -elimination.

US Patent 4,687,765 claims the pharmacological use of LMW-H of molecular weight from 2.000 to 8.000 for
5 lysis of blood thrombi.

US Patent 4,686,288 claims a process for the preparation of mucopolysaccharides having chains consisting of more than 6 saccharide units (molecular weight >1800 daltons) and having 2,5-anhydromannose
10 reducing ends.

The heparin oligosaccharides of the present invention are obtained by an analogous process to the one described in PCT/EP/86/00291 (WO 86/06729). Said process is based on the production of HO. free radicals, by means
15 of divalent metals (e.g. Cu^{++}) and peroxides (e.g. H_2O_2), which are the initiators of heparin depolymerization, See scheme 2. The heparin fragments of the present invention are designed as C 2181I, having mean molecular weight 3.500 daltons, OP 381/1/1, of molecular weight about
20 2.000, C2085II having mean molecular weight 1.700 and OP 381/1/2 of molecular weight 1.000. Said fragments consist of 4÷12 saccharide units and have the following structural characteristics, by which they differ from the prior art products: the end anomeric carbons are reducing
25 and the end manosaccharides consist of N,6-disulfated glucosamine and 2-sulfated uronic acid. This is proved by the signals appearing in the ^{13}C .NMR spectra at 92.7 and 94.4 ppm, respectively. The remaining part of the ^{13}C .NMR spectrum shows that the heparin structure is completely
30 retained. Gel-permeation chromatography shows a conti-

nuous pattern for the heparin oligosaccharides obtained by the present process, differing from a not continuous pattern of 600 daltons, obtained for the oligosaccharides prepared by other deaminative depolymerization processes with nitrous acid. Therefore, the chemical structure of these heparin oligosaccharides is completely different from the one claimed by the other patents.

The oligosaccharides of the present invention have lost the anticoagulant activity of heparin and have antiatherosclerotic activity and antithrombotic activity on both arterial and venous thrombosis; good bioavailability by subcutaneous and oral routes and long lasting effect. They inhibit ex vivo platelet aggregation induced by collagen, ristocetin and ADP. The parent heparin, on the contrary, induces platelet aggregation (Brace L.D., Fareed J., Seminars in Thromb. and Haemost. 11, 190, 1985; Eika C., Scand J. Haematol. 8, 248, 1972) and/or enhances platelet aggregation induced by other agents. The oligosaccharides reduce platelet adhesion. All said activities together could not be predicted on the ground of the searches carried out for EP-A-00291/86.

The pharmacological results hitherto obtained are predictive of the clinical usefulness of the oligosaccharides of the present invention, administered in oral doses of 3-15 mg/kg day, or in form of injectable doses of 1-5 mg/kg day.

The present invention also relates to all the industrial applications of the products of the invention for therapeutical uses in humans as antithrombotic, antiatherosclerotic, fibrinolytic agents having very poor

or no anticoagulant activity. To this purpose, the compounds of the invention are formulated, by means of conventional techniques and excipients, in form of pharmaceutical compositions suitable for parenteral, 5 topic and oral administrations. Examples of formulations suited to parenteral administration comprise sterile solutions in vials. Examples of formulations suited to oral administration comprise capsules, tablets and syrups, in which the active ingredient can be vehicled 10 also in form of liposomes or micelles. Example of formulations for topical administration comprise ointments including the traditional excipients.

The following examples further illustrate the invention without limiting it.

15 **EXAMPLE 1**

30 g of pure dermatan sulfate OP 437 (having $[\alpha]_D^{20} = -60^\circ$; $\text{SO}_3^-/\text{COO}^-$ ratio 1.06 (il farmaco Ed. Pr. 43, 165, 1988), molecular weight $\text{MW}_p = 30.000$ daltons and no anticoagulant activity) are placed in 300 ml of water and 20 300 ml of a 0.5M NaJO_4 solution are added. The solution is kept under stirring overnight. pH is adjusted to 6 and 20 g of NaBH_4 are added portionwise during 3 hours. pH is kept constant to 7 by constant periodic additions of acetic acid. The reaction is thermostated at 25°C . Then 25 the solution is adjusted to pH =3 with 1N HCl and left under stirring for 0.5 hours at room temperature, then pH is adjusted to 5 with N NaOH and the solution is concentrated to half volume. 3 Volumes of methanol are added: a precipitate is obtained which is recovered, 30 redissolved in water, reprecipitated with methanol and

dried to yield 24.7 g of OP 616 (82.3% yield).

EXAMPLE 2

6 g of the product obtained in example 1 are added to 110 ml of water and percolated on 180 ml of an IRA 92 SP resin (Rohm and Haas). The percolated solution is concentrated to small volume and precipitated with 3 volumes of ethanol. Fractions are eluted from the column with 0.25 M, 0.5 M and 3 M NaCl solutions.

After precipitation and drying the following compounds are obtained: 2078 0 M : 2.83 g (47.2% yield); 2078 0.25 M : 0.71 g (11.8% yield); 2078 0.5 M : 0.62 g (10% yield); 2078 3 M : 0.19 g (3% yield).

The characteristics of the obtained compounds compared with the starting product are reported in Table I. The ^{13}C -NMR spectra of fig. 1, recorded on a Varian CFT 20 with 10 mm probe operating at 20 MHz, show in compounds 2078 0 M (fig. 1B) and 2078 0.5 M (fig. 1C) a shift to high fields for the signals assigned to C(2) and C(3) of iduronic and of C(1) of the same residue in comparison with the integral chain dermatan sulfate (fig. 1A). Minor signals are assigned to more sulfated sequences. In fact, the intensity of these minor signals for the "open" dermatan sulfate fractions eluted from an anion exchange resin increases with the increase in NaCl concentrations (fig. 1C).

The obtained compounds show improved antithrombotic activity in comparison with the starting dermatan sulfate (OP437).

EXAMPLE 3

1 g compound 2078 0 M is added to in 100 ml of 0.1 M

HCl and heated to 60°C for 3 hours. The solution is neutralized with NaOH and freeze-dried. Product OP 602 is obtained. The ¹³C-NMR spectrum of this compound (fig. 2) recorded on Bruker AC200 with 5 mm probe operating at 50MHz, shows, in addition to the 12 characteristic signals of the degradation product, two other minor signals marked with an asterisk, one of which, at 91 ppm, is likely due to the "remnant" of glucuronic acid. The signals indicated with A-1, A-2, A-3, A-4, A-5 and A-6 are tentatively assigned to the respective carbons of the galactosamine nucleus. Signal I-4 is assigned to the carbon originally at the 4-position of iduronic acid and now contained in the "remnant". Signal G-4, marked with an asterisk, is assigned to the carbon originally at the 4-position of the glucuronic acid contained in the dermatan sulfate structure. The structure of the degraded product confirms the structure of the compounds obtained and described in example 2.

TABLE 1

	SAMPLES	[α] _D ²⁰	M.W.*	SO ₃ ⁻ /**	APTT ⁺	AXa ⁺⁺	ED50 ⁺⁺⁺
			x10 ⁻³	COO ⁻	U/mg	U/mg	mg/kg
	OP 437	-60°	30	1.06	1	2	3.5
25	2078 O M	- 5°	>22	0.99	1	inactive	1.1
	2078 0.25 M	- 6°	12	1.06	0.8	inactive	1.0
	2078 0.5 M	-13°	22	1.14	1	inactive	0.8

* Molecular weights calculated by HPLC at the distribution peak;

** potentiometrically deduced value;

+ According to Basu D. et al., N. Engl. Med., 287,
324, 1972

5 ++ According to Teien A.N. et al., Thromb. Res., 8,
413, 1976

+++ According to the experimental thrombosis test by
ligature of vena cava, Meyers S. et al., Thromb.
Res., 18, 699, 1980.

EXAMPLE 4

10 Following the procedure described in example 1 and
carrying out the fractionation on an IRA 93SP column (in
the Cl⁻ form) as described in example 2, the following
fractions were obtained :

15 2088 0 M g 5 fractionation yield : 50 %
2088 0.25 M g 0.7 yield : 7%
2088 0.5 M g 1.05 yield : 10.5%

having the characteristics reported in table II below.

TABLE 2

20	[α] _D ²⁰	S%	Uronic Acids %	SO ₃ ⁻ / COO ⁻	M.W. x10 ⁻³	ED50
	2088 0 M	- 6.7°	5.6	33.25	1.02	14.6
	2088 0.25 M	- 7°	5.72	33.38	1.04	9.4
25	2088 0.5 M	- 13°	5.98	33.1	1.1	13.7

EXAMPLE 5 (OP 381/1/1)

300 g of heparin dissolved in 2 l of water and added
with 12 g of copper acetate monohydrate are placed into a
30 reactor. 1000 ml of a 16% hydrogen peroxide are added,

during 3.6 hours, to the reaction mass, keeping pH at 7.5 by means of a N sodium hydroxide solution and raising the temperature from 44°C to 66°C during the first reaction hour, by outer heating.

5 pH is adjusted to 5.5 with 120 ml of 30% acetic acid and 6 g of ascorbic acid are added. The oligosaccharides are cooled and precipitated with 3 volumes of methanol.

 After filtration and drying, 192.7 g of a product are obtained, having mean molecular weight 2.900 daltons.
10 Filtrate A is collected. 191 g of this intermediate product are dissolved in 1.300 ml of water heated to 68°C. 400 ml of a 16% hydrogen peroxide solution are added during 2.3 hours, keeping pH at 7.5 with sodium hydroxide. After cooling, 4 volumes of methanol are
15 added. Precipitate is recovered by filtration and dried. 120 g of a product are obtained. The whole solid material is redissolved in water and percolated on a Chelex 100 (Bio-Rad) resin column (4 cm diameter, 35 cm height). 3 Volumes of methanol are added to the percolate.

20 The precipitate is collected and dried. 99.51 g of a product named OP 381/1/1 are obtained.

¹³C-NMR spectrum of compound OP 381/1/1 shows that the heparin structure is retained with no essential changes, and it also evidences C₁ signals of reducing
25 anomeric carbon atoms of iduronic acid and of the aminosugar. The compound is subjected to in vivo and in vitro biological evaluations (table III) showing a surprising activity in arterial thrombosis (artr. thromb) and venous thrombosis (ven. thromb) experimental models,
30 good bioavailability and a long lasting effect (up to 8

hours after the treatment, when administered subcutaneously).

The s.c. ED₅₀/i.v. ED₅₀ ratio for the oligosaccharides of the present invention is much more favorable than that of heparin. In the kaolin-induced thrombosis model in the rat (Hladovec J., *Physiol. Bohemoslovaca*, 24, 551-554, 1975), i.v. ED₅₀, s.c. ED₅₀ and per os ED₅₀ turned out to be 0.5 mg/kg, 3.6 mg/kg and 9.4 mg/kg, respectively. The ratio of said values, which is an index of product bioavailability, is 1/7/19.

OP 381/1/1, at the concentration of 70 mcg/ml, inhibits platelet aggregation induced by 2 mcg of collagen, ristocetin-induced platelet aggregation and platelet aggregation induced by 16 mcg/ml of ADP in comparison with heparin which, under the same conditions, increases by 20% the ADP-induced aggregation. The product of the invention reduces by 49% platelet adhesion in comparison with physiological saline (in the "Adelplats" test, by Mascia-Brunelli, Hellem A.J., *Scand. J. Haematol.* 7, 374, 1970) causing no thrombocytopenia.

Said characteristics explain the surprising activity of OP 381/1/1 in the arterial thrombosis experimental model.

TABLE 3

Heparin oligosaccharides - Activity

Sample	M.W. (1)	SO ₃ /COO ⁻ (2)	IN VITRO		IN VIVO (ED50 mg/kg)				
			APTT (3) U/mg	AXa (4) U/mg	Art. thromb (5) i.v. s.c.	Ven. thromb (6) i.v. s.c.			
C2081/I	3500	2.19	2	57	3.3	3.6	0.4	1.8	
C2085I	2300	2.16	2	51	2	4.6	0.9	1.8	
OP381/1/1	2000	2.2	1.5	30	1.5	5.9	1.0	2.1	
C2085II	1700	2.2	0.7	9	2.1	4.8	1.2	5	
OP381/1/2	1000		inactiv	2.9	12.2		4.4	21	
Heparin	13500	2.22	170	172	1	8.0	0.2	4.5	
LMW Heparin (7)	4500	2.19	30	78	2		0.3		

(1) By HPLC; (2) according to Mascellani G. et al. Il farmaco Ed: pr; 43, 165, 1988; (3) according to Basu et al. N. Engl. Med. 324, 1972; (4) according to Teien A.N. et al, Thromb. Res., 8, 413, 1976; (5) according to Lavelle S.M. et al. in "Standardization of animal models of thrombosis", Proceedings Ang. Symp. Kitzbühel, H.K.Breddin 1983, 158; (6) according to Reyers et al. Thromb. Res., 18, 699, 1980; (7) product claimed in PCT EP 86/00291.

OP 381/1/2

Filtrate A, which had been collected in the first part of this example 5, is concentrated to 10 liters under vacuum. 3 Volumes of acetone are added: the resulting precipitate is recovered by filtration and redissolved in water (10%) and percolated in 100 ml of Chelex 100 (Bio-Rad) resin. The completely copper free percolate is freeze-dried. 15 g of OP 318/1/2 are obtained. ¹³C-NMR spectrum of compound OP 381/1/2 shows the typical dispersion of signals of very low molecular weight products. Moreover, very evident appear the signals assigned to the reducing anomeric carbons of the aminosugar and of uronic acids. The general heparin structure (which is particularly illustrated by the sulfamino and C₆-sulfate signals) has been retained.

The presence of signals assigned to 3 anomeric carbons is also evident in the region of the anomeric carbons, which presence is generally masked in heparins having higher molecular weights.

EXAMPLE 6 (C 2085)

Fractionation on gel-filtration column.

5 g of the product OP 381/1/1, obtained in example 5, are added to 50 ml of a 0.15 M NaCl solution and percolated in an Ultrogel Ac.A 202 (LKB) column (5 cm diameter, 100 cm height) at 2 ml/min. 420 ml of dead volume are discarded, and 10 ml each fractions are recovered. The first 30 fractions are discarded. Fractions 31 to 104 (740 ml) are combined (I). The 112 other fractions are separately pooled (II).

The two groups of fractions are concentrated to

small volume, salted-off on Trisacryl GF 0.5 M and freeze-dried. The products C 2085 I (3.1 g) and C 2085 II (0.47 g) are obtained. The molecular weight profile is shown in fig. 3c. By way of example, the profiles of the products obtained by depolymerization with nitrous acid, according to example 1 of EP-A-14.184 (fig. 3a) and of the product obtained by depolymerization with nitrous acid according to USA Patent 4.500.519 (fig. 3b) are reported. The integers denote disaccharide units.

10 EXAMPLE 7 (C 2081 I)

According to a process similar to the one of example 6, but collecting only the first 81 fractions, compound C2081 I (1.04 g) is obtained.

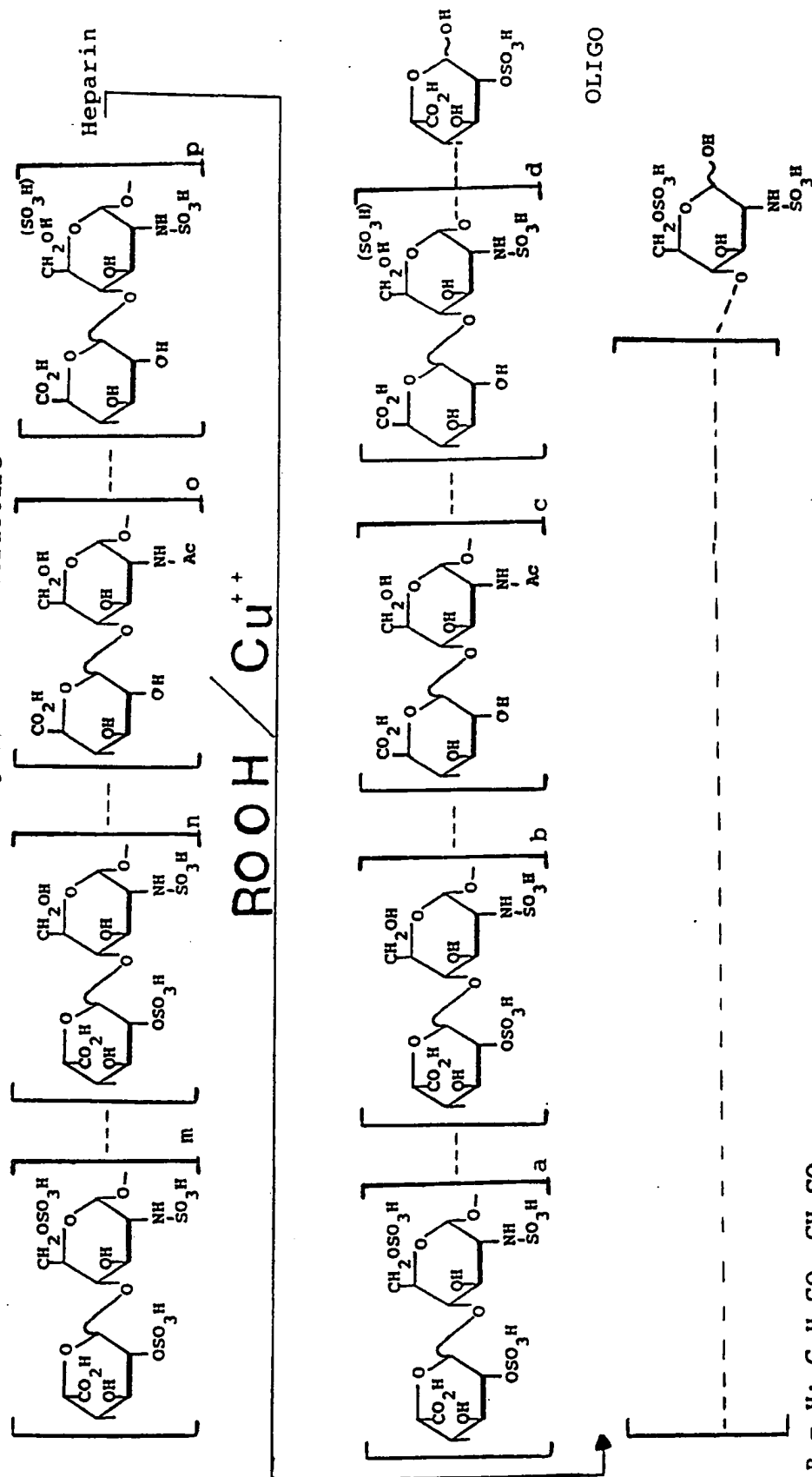
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Scheme 2 - Heparin depolymerization. Oligosaccharide structure

R = H; C₆H₅CO, CH₃CO

Heparin : m ≈ 40% n ≈ 30% o ≈ 10% p ≈ 20%

Oligosaccharides : for a molecular weight of ~1700 Daltons, the statistic abundance

of disaccharides is as follows : a ≈ 1,4 b ≈ 1 c ≈ 0.35 d ≈ 0,7

CLAIMS

1. Open chain dermatan sulfate fractions which can be obtained by means of periodate ions, under conditions for the oxidative cleavage of the C(2)-C(3) bond of unsulfated uronic acids.
2. Fractions as claimed in claim 1, in which the aldehyde groups obtained by the cleavage reaction of the C(2)-C(3) bond of unsulfated uronic acids have been reduced to hydroxymethyl groups.
3. Dermatan sulfate fractions as claimed in claims 1 and 2, further fractionated on anion exchange resins by means of electrolyte solutions and having a $\text{SO}_3^-/\text{COO}^-$ ratio from 0.9 to 1.4.
4. Fractions as claimed in claims 1, 2 and 3, hydrolyzed in acid or alkali medium to fragments having molecular weight to 2.000 daltons.
5. A process for the preparation of dermatan sulfate fractions as claimed in claims 1, 2, 3 and 4, comprising the reaction of dermatans or fractions thereof with an oxidizing agent, such as a periodate ion, the subsequent possible reduction of the aldehyde groups at the C(2) -C(3) positions to hydroxymethyl groups, the possible fractionation of the obtained products on anion exchange resins to separate the fragments of different charge density, and the possible subsequent acid hydrolysis to produce oligosaccharides having the desired molecular weight.
6. Heparin oligosaccharides which can be obtained by radical depolymerization of heparin, containing an average

of 4-12 monosaccharides.

7. Oligosaccharides as claimed in claim 6, containing end groups consisting of 2-O-sulfated iduronic acid or N,6-disulfated glucosamine, which have the reducing anomeric carbon.

8. Heparin oligosaccharides as claimed in claims 6 and 7, having molecular weights of 3.500 to 1.000 daltons and essentially the same $\text{SO}_3^-/\text{COO}^-$ ratio as the one of starting heparin.

9. Heparin oligosaccharides as claimed in claims 6, 7 and 8, having anti-Xa activity until 60 times lower than that of heparin and having high antithrombotic activity even in arterial thrombosis, high anti-platelet aggregation activity, high bioavailability by the subcutaneous and oral routes.

10. Pharmaceutical compositions having high antithrombotic, thrombolytic, antiatherosclerotic activities, containing as the active ingredient the dermatan sulfate fractions and fragments as claimed in claims 1 to 4.

11. Pharmaceutical compositions having antithrombotic, thrombolytic, antiatherosclerotic activities, containing as the active ingredient the heparin oligosaccharides as claimed in claims 6 to 9.

12. Pharmaceutical compositions as claimed in claims 10 or 11, for parenteral, oral and topical administrations, in form of sterile injectable solutions or suspensions, capsules, tablets, syrups, in which the active ingredient can possibly be vehiculated either in liposomes or micelles or ointments or salves.

1/2

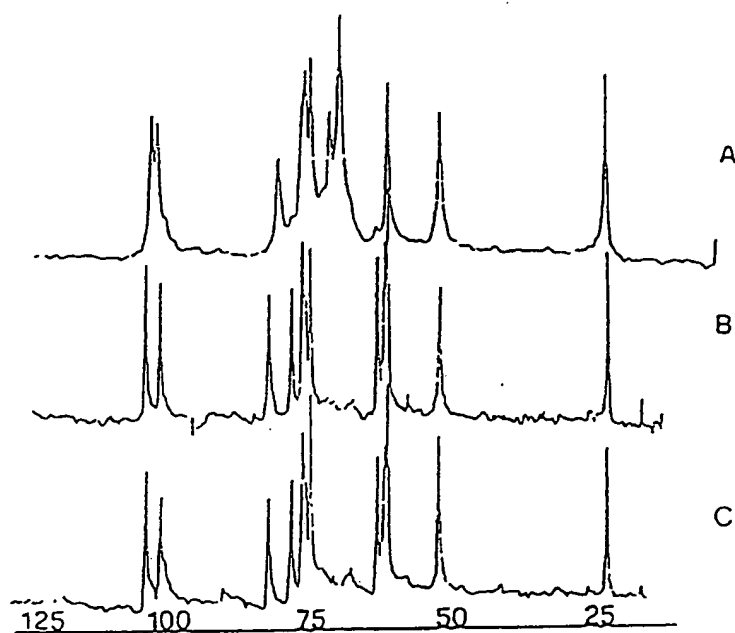
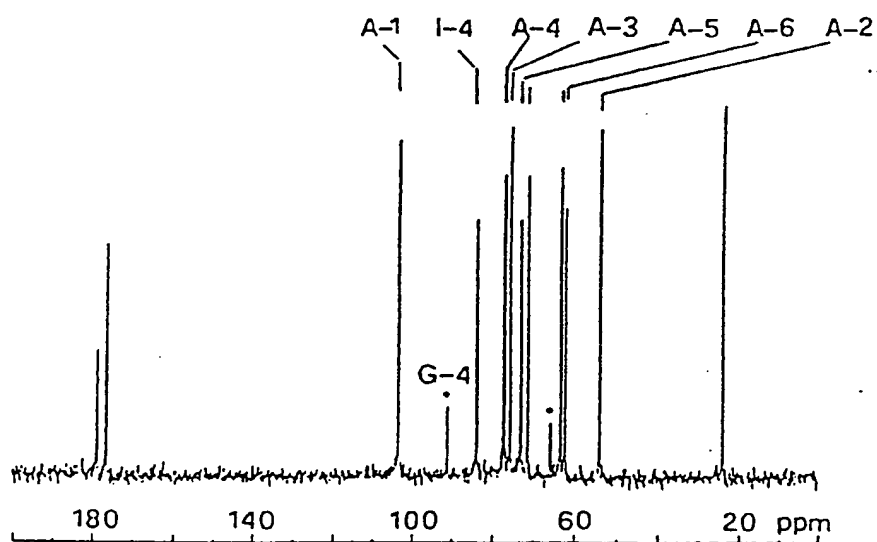
Fig. 1 ^{13}C -NMR Spectra (20 MHz)

Fig. 2

2/2

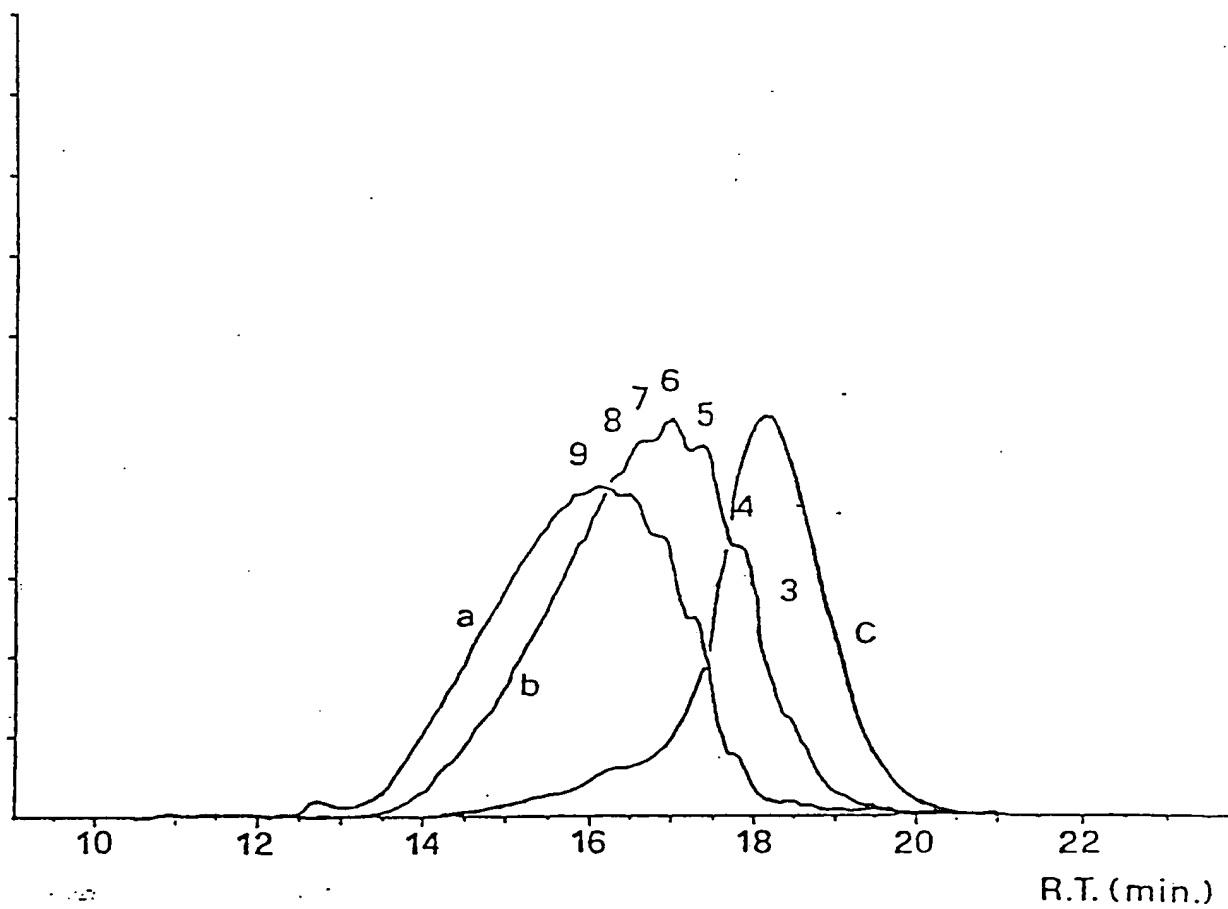


FIG. 3